IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

(17)

8/18/94

Applicant: Malcolm J. Simons

Assignee: GeneType AG

Title: "INTRON SEQUENCE ANALYSIS METHOD FOR DETECTION OF ADJACENT AND REMOTE LOCUS ALLELES AS HAPLOTYPES"

Serial No. 07/949,652 Filed: September 23, 1992

Examiner: Bradley L. Sisson Group Art Unit: 1807

Attorney Docket No.: M-1647-6C US

San Jose, California

THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

DECLARATION

Sir:

I, Pablo Rubinstein, hereby declare the following. I am the Director of Immunogenetics of the New York Blood Center. Attached is a copy of my curriculum vitae.

I previously submitted a Declaration regarding Malcolm Simons' discovery that one could use relatively short regions of non-coding sequences closely linked to a polymorphic gene to define the corresponding coding region allele. At that time, Malcolm Simons had definitively demonstrated that relatively short non-coding region sequences contained informative polymorphisms which can be used as the basis of a complete HLA Class II typing system, because they correlate perfectly with the expressed exonic sequence variations.

At that time, I could see no reason why such correspondence of polymorphic non-coding and coding region sequences should be limited to the HLA genes. However, Malcolm Simons' observations on this correspondence had been tested only in the HLA genes, a unique family of genes which are probably under selective pressure for functional diversity and therefore present a high degree of coding region polymorphism. Conceivably, exon/intron polymorphic variation could be

LAW OFFICES OF
SKJERVEN, MORRILL,
MacPHERSON, FRANKLIN
& FRIEL

25 METRO DRIVE
SUITE 700
SAN JOSE, CA 95110
(408) 283-1222
FAX (408) 283-1233

restricted to highly polymorphic genes under the pressure of selection for variation, and Dr. Simons had not provided evidence that his method would work in polymorphic genes, generally.

Since that time, Malcolm Simons and I have discussed a number of recent articles which demonstrate that the allelic variants of coding regions are accompanied by concordant variants of adjacent non-coding regions of diverse non-HLA genes. This feature of allelic variation is thus found in systems with limited polymorphism in humans and also in other mammals and even in insects.

Most pertinent to the issue are the following reports. Eisses et al., *Mol. Biol. Evol.*, **7**: 459-469 (1990) uncovered the consistency between specific variations of the coding regions of the alcohol dehydrogenase ADH-71k gene in Drosophila (the common fruit-fly) and variations in the sequence of their introns. The phenomenon is thus present in species separated by several hundred million years of evolution.

Messer et al., *J. Exp. Med.*, **173**:209-219 (1991) describe exactly the same situation in the case of the tumor necrosis factor gene in humans. This gene has a few alleles, that is, it is not highly polymorphic. Specific variation in the introns can be found that is concordant with the expressed variation in each case. The phenomenon is, thus, not restricted to highly polymorphic genetic systems.

Brooks et al., *Am. J. Hum. Genet.*, **52**:835-840 (1993) extended the findings of consistent exon-intron variation, to the relatively rare mutant of the aldolase B gene causing fructose intolerance in humans. Here, selective pressures have in all probability weighed against the high frequency of this mutation. Thus, Malcolm Simons' observation applies to genes that have rare deleterious variation, as it does to those in which polymorphism may have functional advantages.

LAW OFFICES OF
SKJERVEN, MORRILL,
MacPHERSON, FRANKLIN
& FRIEL

25 METRO DRIVE
SUITE 700
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MacPHERSON, FRANKLIN
& FRIEL

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SAN JOSE, CA 95110
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LAW OFFICES OF
SKJERVEN, MORRILL,
MacPHERSON, FRANKLIN
& FRIEL

25 METRO DRIVE
SUITE 700
SAN JOSE, CA 95110
(408) 283-1222
FAX (408) 283-1233

Schlieben et al., *Animal Genetics*, 22:333-342 (1991) demonstrated an identical situation for the genetic variants of casein in cattle.

In view of the generality of the phenomenon in so varied a group of genes and species, one would expect that any polymorphic genetic locus would have correlated variation in its coding and non-coding regions. In some cases, detection of a specific genetic variant (or allele) can be greatly facilitated by the detection of such allele-specific non-coding sequence variation.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Aug. 1, 1994

Pablo Rubinstein, M.D.
Director, Immunogenetics

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on August 4 1994

8/4/94
Date of Signature

Attorney for Applicant

LAW OFFICES OF
SILVEVEN, MORELLI,
MacPHERSON, FRANKLIN
& FRIEL

25 METRO DRIVE
SUITE 700
SAN JOSE, CA 95110
(408) 283-1222
FAX (408) 283-1233

CURRICULUM VITAE
Pablo Rubinstein, M.D.

Home Address: 92 Wood Hollow Lane
New Rochelle, New York 10804
Home Telephone: 914-235-5002
Date of Birth: February 26, 1939, Santiago, Chile
Social Security Number: 121-38-7913

University Studies:

1955-1962	Medical School, Faculty of Medicine, Universidad de Chile
1964	Thesis: "Immunogenetics of the H-2 locus of the Mouse", Summa cum Laude

Professional Degree:

Medico-Cirujano, (M.D.) May 1962
Universidad de Chile
ECFMG Certification 1962

Specialized Licenses:

New York State Department of Health, Certificate of Qualification, Clinical Laboratory Director.
New York City Department of Health, Certificate of Qualification, Clinical Laboratory Director.

Post-Graduate Studies:

1962-1963	Resident in Surgery, Hospital Clínico Jose Joaquín Aguirre, Universidad de Chile.
1963-1965	Post-Doctoral Research Fellow, Department of Medical Research, M.I. Bassett Hospital, Cooperstown, Columbia University, New York.
1965-1966	International Post-Doctoral Research Fellowship, U.S. Public Health Service, National Institutes of Health., at the Blood Bank, Department of Hematology, Mount Sinai Hospital, New York, N.Y.

Positions Held:

1978-date	Senior Investigator, Lindsley F. Kimball Research Institute, New York Blood Center, New York, N.Y.
1977-date	Director, Immunogenetics Laboratory, Lindsley F. Kimball Research Institute, New York Blood Center, New York, N.Y.
1976-date	Clinical Professor of Pathology, College of Physicians and Surgeons, Columbia University, New York, N.Y.
1975-date	Associate Medical Director, Greater New York Blood Program, New York Blood Center, N.Y.

1972-1978	Investigator, Lindsley F. Kimball Research Institute, New York Blood Center, N.Y.
1969-1971	Professor, Department of Biology and Genetics, Faculty of Medicine, Universidad de Chile.
1968-1969	Profesor encargado de Curso, Chair of Biology, Faculty of Medicine, Universidad de Chile. (equivalent to Associate Professor).
1967-1971	Director, The Blood Bank, Hospital Clínico José Joaquín Aguirre, Universidad de Chile.
1967-1970	Visiting Professor, Department of Immunohematology and Institute for Radiopathology and Radiation Protection, University Hospital, Leiden, Holland. (two-month Immunogenetics courses each year).
1966	Research Associate, Department of Immunohematology, University Hospital, Leiden. (July-September).
1965-1966	Research Fellow, The Blood Bank, Department of Hematology, The Mount Sinai Hospital, New York, N.Y.
1964	Research Associate, The Jackson Laboratory, Bar Harbor, Maine. (March-June).
1956-1968	Research Associate, Department of Immunogenetics, Chair of Biology and Genetics, School of Medicine, Universidad de Chile.
1956-1962	Instructor, Chair of Biology and Genetics, School of Medicine, Universidad de Chile.

Other National and International Appointments:

1990-1993	Chairman, Scientific Affairs Committee, American Society for Histocompatibility and Immunogenetics.
1988	Chairman, Task Force on the establishment of National Long-Term Resource, National Institute of Diabetes and Kidney Diseases, N.I.H.
1988	Member, National Grant Review Committee, The Juvenile Diabetes Foundation.
1981-1984	Member, Mammalian Genetics Study Section, N.I.H.
1980	Councilor, International Histocompatibility Testing Workshop Organization.
1978	Member, WHO International Committee on Genetic Nomenclature
1978	Member, Diabetes Data Commission
1969	Consultant, Department of Immunohematoloy, University Hospital, Leiden, Holland.
1969	Advisor, Pan American Health Organization (P.A.H.O) in post-graduate medical education.

Scientific Societies:

The Transplantation Society (founding member)
The American Association of Immunologists
American Society for Histocompatibility and Immunogenetics
The American Diabetes Association
The Multiple Sclerosis Society

Editorial Boards:

Human Immunology
Journal of Experimental Medicine

Pablo Rubinstein, M.D.

Publications

1. Pizarro, O., Hoecker, G., Rubinstein, P. and Ramos, A.: The distribution in the tissues and the development of the H-2 antigens in the mouse. Proc. Natl. Acad. Sci. 84:1900, 1961.
2. Pizarro, O., Rubinstein, P., and Hoecker, G.: Properties of histocompatibility H-2 antigens from different tissues. Guy's Hospital Reports 112:392, 1963.
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4. Rubinstein, P.: Different immunogenicity of the H-2 antigens of liver and spleen in mice. Transplantation 2:695, 1964.
5. Rubinstein, P. and Ferrebee, J.W.: The H-2 phenotypes of random-bred Swiss-Webster mice. Transplantation 2:715, 1964.
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8. Rubinstein, P., Puza, A., Vlahovic, S. and Ferrebee, J.W.: Isohemagglutination in dogs: The dextran method. Folia Biol. Praha 10:36, 1964.
9. Rosenfield, R.E., Rubinstein, P., Szymansky, I. and Kochwa, S.: Measurement of erythrocytic antigen-antibody reactions with an AutoAnalyzer. Proc. XI Congr. Intl. Soc. Blood transfusion 11:65, 1966.
10. Cherry, S.H., Goodman, S., Rubinstein, P. and Haber, G.V.: Assay of prenatal Rh antibodies to determine the need for amniotic fluid studies. Prox XI Congr. Intl. Soc. Blood Transfusion 11:33, 1966.
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12. Rosenfield, R.E., Rubinstein, P. and Cherry, S.H.: Erythroblastosis: current concepts. Advances in immunogenetics, T.J. Greenwald, ed., Chapter 6, 1966.
13. Rosenfield, R.E., Rubinstein,P., Dausset, J., Lalezari, P. and van Rood, J.J.: Hemagglutinating activity of anti-leukocyte sera. Vox Sang. 13:461, 1967.
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15. Rubinstein, P., Morgado, F., Ferrebee, J.W. and Blumenstock, D.: Hemagglutinins and skin grafting in dogs. Transplantation 6:961, 1968.
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21. Tellez, A. and Rubinstein, P.: A rapid method for the separation of blood cells. Transfusion 10:223, 1970.
22. Rubinstein, P.: Immunogenetic aspects of the evolution of renal transplants. Rev. Med. Chile 99:147, 1971.
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66. van der Does, J.A., Rubinstein, P. and Meera Khan, P.: A rare PGM-1 variant in Chilean Aymara Indians. Human Genetics 45:327-429, 1978.
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70. Rubinstein, P. and Suciu-Foca, N.: HLA and the genetic aspects of the predisposition to juvenile diabetes mellitus: A follow-up. In: Treatment of early diabetes, R.A. Camerini and B. Hanover, Eds., Plenum Press, 33-40, 1979.
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